Effects of an Endocrine Disruptor on Prostate Development and Growth

Project Scope

The goal of this research project was to determine the consequences of exposure to endocrine disrupters, particularly environmental estrogens, during fetal prostate development. The specific objective of the experiments was to understand the mechanisms associated with our initial finding of dose-dependent effects on prostate growth as a result of fetal exposure to environmental chemicals. Regional growth effects in the urogenital sinus (UGS) were examined following exposure of mouse fetuses to a low, physiologically relevant dose of the pesticide methoxychlor (MXC). The effects of a high dose of MXC have also been examined and were compared to diethylstilbestrol (DES) treatment.

The objectives of this research project were to:

- Examine changes in the regional epithelial growth pattern in the developing fetal mouse prostate in the offspring of pregnant mice receiving environmentally relevant low doses of methoxychlor
- Compare the effects of higher fetal doses of MXC to the effects of diethylstilbestrol (a positive control) during the same developmental stages;
- Characterize the ductal budding patterns and morphometry, androgen and estrogen receptor localization, cell proliferation and programmed cell death in the developing prostate of newborn male mice exposed to MXC or DES *in utero*;
- Characterize the growth and ductal branching morphogenesis, steroid receptors and cell proliferation, in the prostate of mature, middle aged and senescent male mice after exposure to environmental estrogens.

Grant Title and Principal Investigator

Effects of an Endocrine Disruptor on Prostate Development and Growth.

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Key Findings and Implications

- Three-dimensional morphometric reconstruction has been successfully used to evaluate developmental patterns of the urogenital complex and prostate of fetal and adult mice under the influence of environmental estrogens
- The low dose of methoxychlor (MXC) increased the size of the dorsal prostate region and coagulating gland with a similar increase in the tissue volume of the ventral prostate.
- At the high doses, mice treated with MXC or diethylstilbestrol (DES) had a reduced number of prostatic ductal tips, which did not differ between the age groups.
- Morphological changes in the prostate were more apparent in mice at two months compared to one month.
- Anatomical effects on the ducts and the urogenital sinus (UGS) were more readily observed in the 3-D reconstructions compared to measurements of the number of prostatic outgrowths.
- Estrogenic endocrine disruption in fetal mice results in a permanent change in growth parameters that are specific to certain regions of the UGS and associated structures.

Publications include two peer reviewed articles.

Project Period: July 1999 to June 2002

Eight groups of male mice, comprising two controls, plus animals treated prenatally with a low, medium or high dose of MXC and DES, were examined. Prostate tissue was analyzed at four different ages using 3-dimensional (3-D) serial section reconstruction, in situ hybridization to detect steroid receptors, and evaluation of cell proliferation and programmed cell death indices. Pregnant female CD-1 mice were fed oil (control) or oil plus DES (0.1 µg/kg); low-dose MXC (100 µg/kg); and high-dose MXC (100 mg/kg) on gestation days 14 through 18. Caesarian delivery was performed on day 19. To preclude intrauterine

position effects, one 1M male per litter was used for the studies. A total of five animals were taken for each group. The urogenital complex was fixed *in situ* according to previously published protocols. Serial sections were prepared from coded samples. Morphometric analysis was employed to characterize patterns of development and the numbers of ductal buds in the urogenital sinus (UGS). Three-dimensional (3-D) reconstructions were prepared from the control, positive control (DES), and MXC-treated animals (Winsurf, University of Hawaii). The numbers of prostatic ducts developing in specific UGS regions were calculated from the reconstruction data sets.

On the basis of the potential estrogenic effects of MXC, It was hypothesized that the low doses would result in a) an increased number of buds and/or size of the developing prostate, and that this effect would be particularly noticeable in the dorsal region of the prostate; b) a parallel increase in the cell proliferation rate would be seen in the dorsal region; c) an increase in the number of ductal branch points or terminal tips would be seen in adult prostate glands; and d) changes in steroid receptor localization, cell proliferation, and cell death would be seen in the prostate of aging males resulting from endocrine disruptor exposure during fetal development.

Relevance to ORD's Multi-Year Research Plan

This project contributes to ORD's Multi-Year Plan long-term goal of determining the extent of the impact of endocrine disruptors on humans, wildlife, and the environment. This research aims to understand the mechanisms associated with initial findings of dose-dependent effects on prostate growth as a result of fetal exposure to environmental estrogens. The findings providemechanistic insights into the relationship between exposures to environmental estrogens and prostate disease.

Project Results and Implications

The most significant effect of low-dose MXC was an increase in the size of the dorsal prostate region and coagulating gland, in agreement with previous low-dose studies. A similar increase occurred in the tissue volume of the ventral prostate. Estrogenic endocrine disruptors do not normally affect this region, but the results may reflect the fact that MXC may act both as an estrogen agonist and antagonist in different cells. Except for the seminal vesicle and utricle, a consistent pattern of higher UGS tissue volume was seen in the low-dose treated mice. An important finding was the increased size of the ventral mesenchymal pad. This region of mesenchymal tissue is intimately associated with the process of branching morphogenesis in the distal tips of the ventral prostate ducts. Whether the increase in size is a consequence of increased growth stimulation of the stromal cells by a low dose of MXC or a consequence of an increased number of cells in the ductal tip epithelium, remains to be determined. The size of the ventral mesenchymal pad is associated with a specific expression of fibroblast growth factor (FGF-10), which may also be upregulated by MXC exposure. Although there were no significant differences in the number of developing prostatic outgrowths between control and MXC-treated animals, effects of the low-dose MXC were apparent when the volumes of specific UGS regions were analyzed.

Anatomical effects of low-dose MXC exposures on the ducts and the UGS were readily observed in the 3-D reconstructions, and showed differences that were not recognized by examining the number of prostatic outgrowths. The UGS in the region of the prostatic sulci (or furrows) along which the dorsal ducts develop, was much more pronounced in the low-dose MXC- and DES-treated mice than in controls, which confirms previous findings for other low-dose endocrine disruptors. In addition, the volume of the specific UGS structures (dorsal, lateral, ventral, and coagulating glands) was reduced in the high-dose MXC group, likely as a consequence of ductal elongation and narrowing. Temporal and spatial interactions of the prostatic epithelium and surrounding mesenchyme are thought to play an important role in the development of ductal patterning, and these interactions may have been more affected by the high-dose treatment. The long-term consequences of such morphological changes on the adult prostate require further study.

To examine whether the fetal exposure to MXC had a permanent effect on prostate growth, the number of ductal tips was determined in one-month and two-month-old mice by micro-dissection. It was found that

e total numbers of ductal tips at one month of age were similar in the control and low-dose mice. Both DES- and high-dose MXC-treated mice were found to have a reduced number of prostatic ductal tips; the reduction was constant across all age groups. A small increase in the number of ductal tips in the low-dose MXC mice at two months of age appeared consistent with the increase in the number of tips observed in the dorsolateral prostates and the coagulating glands of the two-month-old mice. This finding suggests that the increased volume of these regions in the fetal mice results in a permanent change in prostate growth parameters in certain regions of the UGS caused by estrogenic endocrine disruption. By comparison, the high dose of MXC had an opposite, growth-promoting effect on the ventral ductal system. Additional analyses of these data are in progress.

Microdissection and representative images of the microdissected dorsolateral prostate show that morphological changes were more apparent in the older mice (two months) than in younger animals. No distinct differences between the low-dose DES and low-dose MXC groups were apparent, but at later stages of growth (two months), differences in ductal growth patterns were observed. For example, compared to the pattern of ductal branching in the control dorsolateral ducts (a palmate pattern), the low-dose DES-treated mice exhibited more swollen ductal tips. This is consistent with the increased volumetric changes seen in the dorsolateral prostate of the fetal mice, especially in the most dorsal region.

These results, along with previously published data indicate that endocrine disruption during critical periods of reproductive development in the male fetus are likely to cause alterations of growth parameters in the prostate which may persist into adulthood. The cellular and biochemical nature of these changes in growth patterns needs to be investigated in the regions of the prostate exhibiting the most significant responses to the endocrine disruptors. Implications for human health include insights into effects of endocrine disrupting chemicals on human prostate development and prostate cancer risk associated with permanent changes in growth parameters in the UGS and associated structures.

Investigators

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NCER Project Abstract and Reports:

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/446/report/0